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I, KAY WARD, ACTING MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 2218 for a patent by COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION and RUMENTEK INDUSTRIES PTY LIMITED filed on 13 August 1999.

I further certify that the above application is now proceeding in the name of COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION pursuant to the provisions of Section 113 of the Patents Act 1990

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AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

Feed Supplement for Altering Milk Fat Profile

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This invention is best described in the following statement:

Feed Supplement for Altering Milk Fat Profile

Technical Field

The present invention relates to feeding techniques for designing the nutritional and physico-chemical properties of milk fat derived from ruminants. In particular, it describes feed supplements which produce milk having a desired fatty acid composition, and which is useful in producing products with a range of melting profiles.

Background Art

During the past three decades a range of feed supplements have been developed with the aim of manipulating the fatty acid composition of milk fat. The most effective way to increase the C18 unsaturated fatty acid content of milk fat is to use a feed supplement in which the constituent triacylglycerols are protected from ruminal biohydrogenation by encapsulation in a matrix of aldehyde-treated protein.

Other techniques have also been developed including feeding of full fat rape seed and soybean supplements, heat treated/jet sploded oil seeds, calcium salts of long chain fatty acids, prilled or pelleted fats and butyl soyamide esters. However, there is an enormous variation in the responses observed (Palmquist, *et al.*, 1993), and it can be concluded that these approaches do not provide a reliable and consistent feed supplement to alter the nutritional and physico-chemical properties of milk fat.

In recent times, the specifications of the ideal milk fat from a nutritional and physico-chemical view point have changed dramatically. For example, C18 *cis* monounsaturated fatty acids (oleic acid) have been shown to lower the cholesterol content of human low density lipoproteins (LDL) (Noakes *et al.*, 1996). In contrast, the C18 *trans* monounsaturated fatty acid (elaidic acid) will increase the cholesterol content of LDL in humans (Noakes *et al.*, 1996). In addition, the role of n-3 fatty acids in infant nutrition and in particular their importance in neural development and vision has been recognised (Noakes *et al.*, 1996).

Therefore, the challenge is to design feed supplements that produce milk fat containing a fatty acid composition appropriate for either soft or hard fats. For example, soft fats would be characterised by:

* a reduction in the proportions of saturated acids in particular myristic and palmitic as these two acids significantly elevate human LDL cholesterol and also contribute to "hardness" of milk fat
* an increase in C18 *cis* mono-unsaturated (oleic) without increasing C18 *trans* mono-unsaturated (elaidic)
* an increase in C18 di-unsaturated (C18:2), including conjugated isomers
* an increase in C20 and C22 omega fatty acids, that is, C20:5 and C22:6 respectively
* an increase in C18 tri-unsaturated (C18:3)

Conversely, harder milk fats would be characterised by:

* high proportions of saturated fats

* increases in C16:0 and C18:0.

By altering the amount and type of protected lipid fed, it is possible to produce milk products with a wide spectrum of physical characteristics. This could reduce or eliminate the need for expensive fractional crystallisation and enzymatic inter-esterification procedures that are currently being used to improve the physical and nutritional properties of milk fat.

The present invention describes the use of nutritional materials that are protected against rumen degradation and provide a feed supplement which produces milk fat with the above specifications.

Object of the Invention

An object of the invention is to provide a method for altering the fatty acid profile of milk from ruminant livestock to obtain milk comprising desired proportions and/or types of fatty acids.

Disclosure of the Invention

According to a first embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method comprises feeding to the female ruminant livestock protected lipid having said desired proportions and/or types of fatty acids, such that about 60 to about 90% of said protected lipid is capable of passing through the rumen undigested leaving about 60 to about 90% of said protected lipid available for digestion post-ruminally.

According to a second embodiment of the invention there is provided a protected lipid, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected lipid is such that about 60 to about 90% of said protected lipid is capable of passing through the rumen of ruminant livestock undigested, leaving about 60 to about 90% of said protected lipid available for digestion post-ruminally.

According to a third embodiment of the invention there is provided use of a protected lipid, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected lipid is such that about 60 to about 90% of said protected lipid is capable of passing through the rumen of ruminant livestock undigested, leaving about 60 to about 90% of said protected lipid available for digestion post-ruminally.

It is preferred that about 75 to about 90% of protected lipids are capable of passing undegraded through the rumen.

According to a fourth embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method comprises feeding to the female ruminant livestock, protected lipid having said desired proportions and/or types of

fatty acids, wherein said protected is lipid produced by the emulsification of lipid with protein in the presence of between about 1.5 grams to about 3.0 grams of formaldehyde per 100 grams crude portion.

According to a fifth embodiment of the invention there is provided a protected lipid having desired proportions and/or types of fatty acids, when used in altering the fatty acid profile of milk from female ruminant livestock to have said desired proportions and/or types of fatty acids, wherein said protected is lipid produced by the emulsification of lipid with protein in the presence of between about 1.5 grams to about 3.0 grams of formaldehyde per 100 grams crude portion.

According to a sixth embodiment of the invention there is provided use of a protected lipid having desired proportions and/or types of fatty acids, in the preparation of feed for altering the fatty acid profile of milk from female ruminant livestock to have said desired proportions and/or types of fatty acids, wherein said protected is lipid produced by the emulsification of lipid with protein in the presence of between about 1.5 grams to about 3.0 grams of formaldehyde per 100 grams crude portion.

It is preferred that the protected lipid is produced by the emulsification of lipid with protein in the presence of between about 2.0 grams to about 3.0 grams of formaldehyde per 100 grams crude portion.

Preferably, the protected lipid fed in accordance with the first and/or second embodiments of the invention does not constitute the entire ration, but may be fed together with any other source of processed or unprocessed feedstuff.

Typically, in one aspect of the invention, the desired proportions and/or types of fatty acids in the altered fatty acid profile of the milk fat reflects a softer fatty acid profile, wherein the softer fatty acid profile is a consequence of a milk fat containing less saturated and more unsaturated fatty acids (desired proportions of fatty acids), which itself is produced by feeding female ruminant livestock protected lipid comprising less saturated and more unsaturated fatty acids (desired proportions of fatty acids). Even more typically, the protected lipid as fed, and as a consequence, the fatty acid profile of milk so produced, comprises the following proportions of fatty acids: C18:1 cis (25-45%w/w), C18:2 (4-15%w/w) and C18:3 (1-8%w/w). Still more typically, the protected lipid as fed, and as a consequence, the fatty acid profile of milk so produced comprises the following proportions of fatty acids: C18:1 cis (30-40%w/w), C18:2 (6-10%w/w), including conjugated isomers (0.5 to 5%), C18:3 (1-4%w/w) and C20 and C22 omega fatty acids, C20:5 and C22:6, (1-2%w/w).

Typically, in another aspect of the invention, the desired proportions and/or types of fatty acids in the altered fatty acid profile of the milk reflects a milk having a harder fatty acid profile, which is itself produced by feeding female ruminant livestock protected lipid comprising a harder fatty acid profile (desired proportions of fatty acids). More typically, the fatty acid profile comprises milk fat containing more saturated and less unsaturated fatty acids, which is again produced by feeding female ruminant livestock protected lipid

comprising more saturated and less unsaturated fatty acids. Even more typically, the protected lipid as fed, and as a consequence, the fatty acid profile of milk so produced, comprises the following proportions of fatty acids: 25-35%w/w C16:0, 20-30%w/w C18:0 and 20-25%w/w C18:1.

Typically, the term "fatty acid profile" describes the particular fatty acid constituents of milk obtained from female ruminant livestock fed protected lipid comprising the particular fatty acid constituents. In one aspect, a particular fatty acid profile may reflect milk containing a high proportion of soft fats, and such fats would be characterised by the following: reduction in the proportions of saturated acids in particular myristic and palmitic, as these two acids significantly elevate human LDL cholesterol and also contribute to the "hardness" of milk fat; an increase in C18 *cis* mono-unsaturated (oleic) fatty acids without increasing C18 *trans* mono-unsaturated (elaidic) fatty acids; an increase in C18 di-unsaturated (C18:2) fatty acid, including conjugated forms of linoleic acid; an increase in C18 tri-unsaturated (C18:3) fatty acid; and/or an increase in C20 and C22 omega unsaturated fatty acids, such as, C20:5 and/or C22:6.

Conversely, a particular fatty acid profile may reflect milk containing a high proportion of hard fats, obtained from female ruminant livestock fed protected lipid comprising a high proportion of hard fats. Further, such fats would be characterised by: high proportions of saturated fats and increases in the relative proportions C16:0 and C18:0 fatty acids.

In general, the protected lipid is as described in Australian Patent Nos. 450 530 and 659 557, the disclosures of which are incorporated herein by reference.

According to a seventh embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids in accordance with the first or fourth embodiments of the invention, wherein said method further comprises simultaneously feeding to the female ruminant livestock protected protein, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally.

According to an eighth embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising protected protein, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally.

According to a ninth embodiment of the invention there is provided use of a protected lipid in accordance with the third or sixth embodiments of the invention, further comprising protected protein, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty

acids, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally.

Still more typically, 70-75% of protected protein is capable of passing undegraded through the rumen.

According to a tenth embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids in accordance with the first or fourth embodiments of the invention, wherein said method further comprises simultaneously feeding to the female ruminant livestock protected protein, wherein said protected protein is produced by the reaction with between 0.05g and 1.0g of formaldehyde per 100g crude protein.

According to an eleventh embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising protected protein, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected protein is produced by the reaction with between 0.05g and 1.0g of formaldehyde per 100g crude protein.

According to a twelfth embodiment of the invention there is provided use of protected lipid in accordance with the third or sixth embodiments of the invention, further comprising protected protein, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected protein is produced by the reaction with between 0.05g and 1.0g of formaldehyde per 100g crude protein.

More typically, the protected protein is produced by the reaction with between 0.4g and 0.9g of formaldehyde per 100g crude protein.

In general, the protected protein is as described in Australian Patent No. 659 557, the disclosure of which is incorporated herein by reference.

According to a thirteenth embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids in accordance with the first or fourth embodiments of the invention, wherein said method further comprises simultaneously feeding to the female ruminant livestock protected carbohydrate, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

According to a fourteenth embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising protected carbohydrate, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, such that about 30 to about 80% of said protected carbohydrate is capable of passing

through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

According to a fifteenth embodiment of the invention there is provided use of a protected lipid in accordance with the third or sixth embodiments of the invention, further comprising protected carbohydrate, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

It is preferred that about 50 to about 80% of protected carbohydrates are capable of passing undegraded through the rumen.

According to a sixteenth embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids in accordance with the first or fourth embodiments of the invention, wherein said method further comprises simultaneously feeding to the female ruminant livestock protected carbohydrate, wherein said protected carbohydrate is produced by the reaction with between 0.1 grams and 3 grams of formaldehyde per 100 grams carbohydrate.

According to a seventeenth embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising protected carbohydrate, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected carbohydrate is produced by the reaction with between 0.1 grams and 3 grams of formaldehyde per 100 grams carbohydrate.

According to an eighteenth embodiment of the invention there is provided use of a protected lipid in accordance with the third or sixth embodiments of the invention, further comprising protected carbohydrate, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected carbohydrate is produced by the reaction with between 0.1 grams and 3 grams of formaldehyde per 100 grams carbohydrate.

It is preferred that the protected carbohydrate is produced by the reaction with between 0.5 grams and 2.5 grams of formaldehyde per 100 grams carbohydrate.

According to a nineteenth embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids in accordance with the first or fourth embodiments of the invention, wherein said method further comprises simultaneously feeding to the female ruminant livestock (i) protected protein, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally, and (ii) protected carbohydrate, such that about 30 to about 80% of said protected carbohydrate is

capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

According to a twentieth embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising (i) protected protein, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally, and (ii) protected carbohydrate, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids.

According to a twenty-first embodiment of the invention there is provided use of a protected lipid in accordance with the third or sixth embodiments of the invention, further comprising (i) protected protein, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally, and (ii) protected carbohydrate, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids.

According to a twenty-second embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids in accordance with the first or fourth embodiments of the invention, wherein said method further comprises simultaneously feeding to the female ruminant livestock (i) protected protein, wherein said protected protein is produced by the reaction with between 0.05g and 1.0g of formaldehyde per 100g crude protein, and (ii) protected carbohydrate, and wherein said protected carbohydrate is produced by the reaction with between 0.1 grams and 3 grams of formaldehyde per 100 grams carbohydrate.

According to a twenty-third embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising (i) protected protein, wherein said protected protein is produced by the reaction with between 0.05g and 1.0g of formaldehyde per 100g crude protein, and (ii) protected carbohydrate, and wherein said protected carbohydrate is produced by the reaction with between 0.1 grams and 3 grams of formaldehyde per 100 grams carbohydrate, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids.

According to a twenty-fourth embodiment of the invention there is provided use of a protected lipid in accordance with the second or fifth embodiments of the invention, further comprising (i) protected protein, wherein said protected protein is produced by the reaction with between 0.05g and 1.0g of formaldehyde per 100g crude protein, and (ii) protected carbohydrate, wherein said protected carbohydrate is produced by the reaction with between 0.1 grams and 3 grams of formaldehyde per 100 grams carbohydrate, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids.

According to a twenty-fifth embodiment of the invention there is provided a milk fat obtained from a female ruminant animal fed in accordance with the method of any one of the first, fourth, seventh, tenth, thirteenth, sixteenth, nineteenth or twenty-second embodiments of the invention, or obtained from a female ruminant animal fed a protected lipid in accordance with the second, fifth, eighth, eleventh, fourteenth, seventeenth, twentieth or twenty-third embodiments of the invention, or obtained from a female ruminant animal fed a feed prepared in accordance with the use of any one of the third, sixth, ninth, twelfth, fifteenth, eighteenth, twenty-second or twenty-fourth embodiments of the invention.

More typically, the milk fat in accordance with the twenty-fifth embodiment of the invention is either a soft or hard fat.

Typically, the milk fat obtained in accordance with the twenty-fifth embodiment of the invention is used in the production of milk based products. More typically, the milk based products may be selected from the group consisting of: milk, butter, cheese, yoghurt, chocolate and infant formula. Even more typically, the milk based product is butter having an altered spreadability.

Brief Description of the Drawings

Figure 1 illustrates a graphic representation of the role of feedstuffs, including protected lipids, in altering the proportions of fatty acids in milk. Figure 1 illustrates the differences in melting profiles between softer milk fats and normal milk fats.

Figure 2 also illustrates a graphic representation of the role of protected lipids, in altering the proportions of fatty acids in milk, that is, producing harder milk fats.

Definitions

In the context of the present invention, the following terms have the meanings set out below.

In this specification the term "simultaneously" is used to mean feeding of the ruminant livestock within a period of about 24 hours, that is, to realise the benefits of one embodiment of the invention it is not essential that the intake of protected lipid and protected protein, and/or protected carbohydrate takes place at the same time, rather it is important that within a given 24 hour period the animals blood plasma is enriched with

lipid, protein and/or carbohydrate constituents by absorption from the abomasum or lower digestive tract.

By "protected" we mean treated so as not to be fully exposed to the degradative action of the ruminant environment, but available for absorption from the abomasum or lower digestive tract. Lipids are protected by their encapsulation in a matrix of aldehyde treated protein. Importantly, the degree of protection of the formaldehyde-treated protein encapsulating the lipid is much greater than the degree of protection afforded the protein alone. That is, the availability of the protein protecting the lipid is sacrificed to a large extent in order to maintain the lipid beyond the rumen. Thus ensuring that almost all the protected lipid does indeed pass through the rumen undigested. For the purposes of this invention dietary lipids can be protected from ruminal metabolism by encapsulation in a matrix of cross-linked proteins, and the preferred window of protection ranges from 60% to 90%, especially 75 to 90%. In terms of the protected protein constituent of feed of the invention, the optimal degree of rumen protection lies in the range 60 to 80%, that is, 60 to 80% of the protein content of the supplement will pass undegraded through the rumen. Similarly, in terms of the protected carbohydrate constituent of feed of the invention, the optimal degree of rumen protection lies in the range 30 to 80%, that is, 30 to 80% of the carbohydrate content of the supplement will pass undegraded through the rumen. Suitable techniques should allow accurate control of the amount of cross-linking that occurs between the carbohydrate and protein feedstuffs, and the aldehyde; this may be achieved by varying the amount of aldehyde relative to the carbohydrate and protein content and its amino acid composition, so that the carbohdydrate and protein is optimally "protected" from rumen degradation, but may be completely digested and absorbed from the small intestine.

By "grain" we mean plant derived concentrates, and these include barley, wheat, oats, sorghum etc.

By "carbohydrate" we mean complex carbohydrates such as polyhydroxy aldehydes, ketones, alcohols or acids, their derivatives, and any compound that may be hydrolysed to these.

"Protein" is defined as proteinaceous material containing individual amino acids linked together.

"Fat" is defined as lipid soluble material and normally contains long chain fatty acids of carbon chain length >C10.

By "roughage" we mean plant derived cellulose materials containing varying proportions of fibre which are digested at different rates in the rumen.

By "minerals and vitamins" we mean supplement of anions, cations, trace elements and fat-soluble vitamins A, C, D and E that are normally included in feed rations.

"Protected lipid" is defined as lipid soluble material that normally contains long chain fatty acids and is treated either chemically or physically to reduce its degradation in the rumen, but allows the fatty acids to be available for absorption from the intestine.

The degree of protection ranges from about 60 to about 90%, that is, about 60 to about 90% of the fat supplement will pass undegraded through the rumen. In the context of the present invention when protected lipid is fed, a degree of protection of about 75 to about 90% is preferred.

"Protected protein" is defined as proteinaceous material that is treated chemically or physically to reduce the rate of degradation of the constituent amino acids in the rumen. The degree of protection will vary from about 60 to about 80%, that is, about 60 to about 80% of the protein will pass undegraded through the rumen. In the context of the present invention when protected protein is fed, a degree of protection of about 70 to about 75%, is preferred.

"Protected carbohydrate" is defined as carbohydrates or carbohydrate containing material that is treated chemically or physically to reduce the rate of degradation in the rumen but allows the carbohydrate to be readily digested in the small intestine. The degree of protection will vary from about 30 to about 80%, that is, about 30 to about 80% of the carbohydrate will pass undegraded through the rumen. In the context of the present invention when protected carbohydrate is fed, a degree of protection of about 50 to about 80% is preferred.

Best Modes of Carrying Out the Invention

In the performance of this invention in general, protected lipid is included in the ration at about 20% of dry matter intake. However, it is likely to be most practical to feed animals protected lipid as a supplement which also combines both a protected protein and a protected carbohydrate. Typically these supplements will be fed at the rate of about 20%-40% of the total ration. If protected lipids are used in combination with protected carbohydrate and protected protein, a ratio of 1:3:1 w/w/w is used to manufacture the protected feed supplement and it is included in the ration at about 20-25% during the lactation phase.

An economically viable source of carbohydrate, protein and lipid is likely to be cereal grain. Sources of such cereal grain are likely to include: barley, maize, oats, wheat, rice, millet, triticale, rye, and sorghum. Other sources of lipid, carbohydrate and protein include oil seed, oil and lipids, derived from plants, animals and the by-products of food processing for human consumption. As described by Kirk-Othmer (1980), sources of such oilseeds, oil and lipids include the following: corn, soybean, cotton, lupin, peanut, sunflower, canola, sesame seed oil, olive oil, copra and coconut oil, palm kernels and palm oil, casein, butterfat, lard, fish oils, linseed and oil, tung oil, tallow and yellow grease. A still further source of lipid includes lipid products or conjugated linoleic acid products, derived from oil sources via chemical/biological processes, including, alkali isomerisation techniques.

The wide diversity of lipid sources offers the flexibility to select components of the lipid, carbohydrate or protein according to the relative prices and availability of raw

materials. There is no particular inherent advantage provided by feeding any one lipid, carbohydrate or protein source which precludes its use over another, provided that the source of lipid is such that it produces the desired proportions of fatty acids in the milk products.

Clearly the benefits possible from practising this invention can be expected to be related to the continuity and period of feeding the protected carbohydrate and to amounts fed, but other factors such as cattle specifications, eg. genotype, age, and physiological condition and the environmental situation (temperature, humidity), should also be taken into account when deciding on the feeding regime to be adopted.

The selection of the source of the lipid, carbohydrate and/or protein to be protected, is dependent on their seasonal availability and price. There is no particular inherent advantage provided by any one lipid, carbohydrate and/or protein source which precludes its use over another, provided that the source of lipid is such that it produces the desired proportions of fatty acids in the milk products.

Preferably the protected feed supplement is included in the ration at about 10-45% of dry matter intake during the lactation phase.

More preferably the protected feed supplement is included in the ration at about 15-30% of dry matter intake during the lactation phase.

Even more preferably the protected feed supplement is included in the ration at about 20-25% of dry matter intake during the lactation phase.

Preferably, the supplements are fed at a rate of between 3 and 5 kilograms per ruminant animal per day.

More preferably, the supplements are fed at a rate of between 4 and 5 kilograms per ruminant animal per day.

In one aspect, the present invention provides a method for producing softer milk fat containing less saturated and more unsaturated fatty acids, which comprises the feeding of canola/soybean oilseed supplement in ratios of about 7:3 (w/w) protected from ruminal degradation.

Preferably, the softer milk fat obtained via the feeding regime of the present invention may contain the following proportions of fatty acids: C18:1 cis (25-45%w/w), C18:2 (4-15%w/w) and C18:3 (1-8%w/w).

Even more preferably, the softer milk fat obtained via the feeding regime of the present invention may contain the following proportions of fatty acids: C18:1 cis (30-40%w/w); C18:2 (6-10%w/w), including proportions (0.5 to 5%) of conjugated isomers, C18:3 (2-4%w/w); C20:5 and/or C22:6 (1-2% w/w).

In another aspect of the invention, the softer milk fats may be obtained through the feeding of protected canola seed, sunflower seed, or any other oleyl or linoleyl oil containing oil seed, that is fats containing C18 monounsaturated or polyunsaturated fats. For example, lipids high in C18:1, C18:2 and C18:3 fatty acids.

Furthermore, the softer milk fats may be obtained through the feeding of protected fish oils. For example, lipids high in C20 or C22 polyunsaturated fatty acids, such as C22:5 and/or C22:6 fatty acids.

In a further aspect of the invention, there is provided a method for producing harder milk fat containing more saturated and less unsaturated fatty acids, which comprises for example the feeding of cotton oilseed supplements protected from ruminal degradation.

Preferably, the harder milk fat obtained via the feeding regime of the present invention may contain the following proportions of fatty acids: 25-35%w/w C16:0, 20-30%w/w C18:0 and 20-25%w/w C18:1.

In another aspect of the invention, the harder milk fats may be obtained through the feeding of protected oils enriched in saturates, for example hydrogenated fats.

Preferably, the harder milk fats may be obtained through the feeding of protected cotton seed, due to the presence of cyclopropene fatty acids and additional dietary C18:2 which acts to inhibit Δ9 desaturase enzyme, an enzyme which converts additional C18:0 into C18:1 within the mammary gland.

The milk fat produced by the feeding regime of the present invention may be used in all milk based products, including for example: milk, butter, cheese, yoghurt, chocolate and infant formulas.

Milk based products with the fatty acid characteristics obtained through the feeding regime of the present invention, such as for example: butter, cheese, yoghurt, chocolate and infant formulas, are produced according to the relevant manufacturing processes well accepted in the art.

Preferably, butter derived from the softer milk fat produced by the feeding regime of the present invention provides improved spreadability.

Preferably, milk based products with the fatty acid characteristics obtained through the feeding regime of the present invention contain a desirable ratio of n-6/n-3 fatty acids for human nutrition.

In accordance with the invention, protected lipid, fed together with protected protein and protected carbohydrate, in addition to designing milk fat profiles, the present invention also results in improvements in relation to growth rate and carcass quality.

Test Methods

1. *In-Vitro* Biological Evaluation of Feed Supplements

(a) Ruminal hydrogenation of unsaturated lipids.

Samples of unsaturated lipid supplements (containing ca. 40-50mg of oil) are incubated in test tubes with 10mL of strained rumen fluid. The tubes are flushed with nitrogen, capped with rubber serum caps and incubated in a shaking water bath at 38°C for periods up to 20h. The incubated and corresponding unincubated reaction mixtures are saponified and the fatty acids extracted and methylated. The methyl esters are analysed by gas liquid chromatography (GLC), and the extent of protection against ruminal hydrogenation calculated using the formula:

$$\text{Protection (\%)} = \frac{\text{\% 18:2 after incubation}}{\text{\% 18:2 before incubation}} \times 100$$

The endogenous level of polyunsaturated fatty acids in the rumen fluid was always less than 2% by weight of the total fatty acid, and thus had little effect on the above calculations. The hydrogenating capacity of each batch of rumen fluid is verified by incubating the rumen fluid with samples of polyunsaturated oil-casein supplements prepared without formalin.

(b) Ruminal lipolysis of triacylglycerol

Samples of the lipid supplements (containing ca. 40-50mg of lipid) are incubated with 10mL of strained rumen fluid as described above. When the extent of triacylglycerol (TG) hydrolysis is measured by GLC, heptadecanoic acid (17:0)(20mg) is added to each reaction tube as an internal standard.

The incubated and corresponding unincubated reaction mixtures are extracted with 10mL of chloroform-methanol (C/M 2:1 v/v) containing 0.5mL of 5M HCl. The mixtures of rumen fluid and acidic C/M are vigorously shaken and allowed to stand for 2-4h until two phases were clearly distinguished.

The upper aqueous phase is removed and discarded and the lower organic phase filtered to remove suspended matter. The filtrate is evaporated to dryness using rotary film evaporator, and the extent of TG hydrolysis estimated using either thin layer chromatography (TLC), or if 17:0 was added, GLC methods described below.

(i) TLC analysis of the extracted lipids is carried out using silica gel G and a solvent system of petroleum ether: diethyl ether:acetic acid (84:15:1, v/v/v). The separated lipids are visualised by spraying with an ethanolic solution of 2,7-dichlorofluorescein (0.2% w/v) and viewing under UV light. The extent of TG hydrolysis can only be estimated qualitatively by comparing the relative intensities and sizes of the TG and free fatty acid (FFA) spots in both the incubated and the unincubated reaction mixtures.

(ii) GLC analysis is used in conjunction with the 17:0 internal standard to assess the degree of TG lipolysis. This method relies on the determination of the proportion of 17:0 in the FFA fraction of the incubated and the unincubated lipid extracts. The dilution of 17:0 in the FFA fraction which occurs during incubation is used as an index of ruminal lipolysis. The FFA in the lipid extracts are methylated with diazomethane and the methyl esters separated by GLC. In addition, samples of the total lipid extracts are saponified, acidified, and extracted with petroleum ether, and the total fatty acids obtained are also methylated with diazomethane and analysed by GLC. The GLC 17:0 measurements were used to estimate the following values:

TFA t_0 = Total fatty acids at 0h
TFA t_{20} = Total fatty acids at 20h
FFA t_0 = Free fatty acids at 0h
FFA t_{20} = Free fatty acids at 20h

EFFA t_0 = Endogenous ruminal free fatty acids at 0h (from unincubated rumen fluid controls)

EFFA t_{20} = Endogenous ruminal free fatty acids at 20h (from incubated rumen fluid controls).

From these values it was possible to calculate the following two other values:

RFA t_0 (released fatty acids at 0h) = FFA t_0 - EFFA t_0

and

RFA t_{20} (released fatty acids at 20h) = FFA t_{20} - EFFA t_{20}

The resistance to ruminal lipolysis is then calculated using the formula:

$$\text{Resistance (\%)} = \frac{\text{TFA } t_{20} - \text{RFA } t_{20}}{\text{TFA } t_0 - \text{RFA } t_0} \times 100$$

(c) Ruminal carbohydrate protection

The protection of carbohydrate is determined by the measurement of the residual starch remaining after 24h *in sacco*. 5g of treated or untreated carbohydrate are sealed into 3x5cm nylon bags (52ìm pore size) which are inserted with appropriate weights in the rumen of a sheep for 24h. These bags are removed, washed in deionised water and freeze dried and the weight of residue remaining determined. The residues and incubated samples are ground through a mill (containing a 0.5mm screen) and the starch determined on a 100mg sub-samples enzymatically using a "Megazyme" total starch assay kit (distributed by Deltagen Australia, 31 Wadhurst Drive, Boronia, Victoria Australia, 3155). All starch values measured are corrected to known standards provided in the kit. The protection of the protected carbohydrate is then calculated as the ratio of the total starch in the untreated and treated sample.

(d) Ruminal protein solubility

The release of ammonia during *in vitro* incubation with rumen fluid is used as a measure of the solubility of the proteins. To 10mL of strained rumen fluid, sufficient lipid supplement is added to supply 75mg of protein, and the mixture was incubated anaerobically at 37°C for 20h. The reaction flasks including rumen fluid blanks are treated with 5mL of 0.2 M H_2SO_4. The mixtures are centrifuged to remove suspended matter, and ammonia is estimated in the supernatant after steam distillations. Net ammonia production is calculated from the difference between the incubated and blank values corrected for ammonia initially present.

2. *In-vivo* Biological Evaluation of Supplements

(a) Ruminal carbohydrate protection

The protection of carbohydrate is determined by the measurement of the residual starch remaining after 24h *in sacco*. 5g of treated or untreated carbohydrate are sealed into 3x5cm nylon bags (52µm pore size) which are inserted with appropriate weights in the rumen of a sheep for 24h. These bags are removed, washed in deionised water and

freeze dried and the weight of residue remaining determined. The residues and incubated samples are ground through a mill (containing a 0.5mm screen) and the starch determined on a 100mg sub-samples enzymatically using a "Megazyme" total starch assay kit (distributed by Deltagen Australia, 31 Wadhurst Drive, Boronia, Victoria Australia. 3155). All starch values measured are corrected to known standards provided in the kit. The protection of the protected carbohydrate is then calculated as the ratio of the total starch in the untreated and treated sample.

(b) Ruminal hydrogenation of unsaturated lipids.

This technique is dependent on evidence that the total long chain fatty acids passing from the abomasum is approximately equal to the intake in the diet. Hence the change in concentration of 18:2 and 18:3, gives an approximation of the degree of hydrogenation. The animals are fed basal diets of chopped alfalfa hay and oats (1:1, w/w) 800g/day. The abomasal digesta is sampled via an abomasal fistula at various time periods and ca. 20mL of digesta saponified and fatty acids extracted as described for the rumen fluid incubations. The extracted fatty acids are methylated and analysed by GLC. The proportion of polyunsaturated fatty acid (eg., 18:2) in the abomasal lipids is compared with a theoretical level estimated by assuming (a.) that all of the 18:2 in the lipid supplement was protected against ruminal hydrogenation; (b.) that all of the 18:2 in the basal diet was hydrogenated; and (c.) that there was no significant synthesis or degradation of the carbon skeleton of fatty acids by micro-organisms. The *in vivo* protection of these supplements is calculated using the formula:

$$\% \text{ protection} = \frac{\text{Actual \% 18:2 in abomasum}}{\text{Theoretical \% 18:2 in abomasum}} \times 100$$

As an example, a sheep receiving 400g of alfalfa hay, 400g of crushed oats and 300g of a formaldehyde treated safflower oil/casein (2:1 w/w) supplement would receive 3% of the basal diet of alfalfa and oats as fatty acids, ie., 24g, and 178g of fatty acids from the lipid supplement (corrected for glycerol moiety).

The 18:2 content of the supplementary fatty acids is 75% or 134 g. Using the above assumptions, the content of 18:2 in the abomasal fatty acids should be 134/(178 + 24) = 66%. If the actual 18:2 content of abomasal fatty acids is 53%, then the percentage protection $= \frac{53}{66} \times 100 = 80\%$.

3. Other Chemical Analyses

Moisture content of feed ingredients is determined by heating at 100°C for at least 12h. Protein content is determined by the Kjeldahl method. Formaldehyde content of supplements is determined by the method of Van Dooren J. Sci. Food Agric. (1975). 26: 1263.

The invention will now be described in greater detail by reference to specific to examples, which should not be construed as limiting on the scope thereof.

Examples

Example 1: Feed Supplements for the Production of Softer Fats

Feeding to lactating cows a Canola/Soybean Blend (7:3w/w) supplement at the rate of approximately 10% of dry matter intake, provides 750 g (approximately) fat with the following fatty acid composition (% w/w).

Table 1: Canola/Soybean Supplement (7:3 w/w)

Fatty Acid	% by Wt	g/d
18:1	51.2	345.6
18:2	28.7	193.7
18:3	10.7	72.2

Example 2: Feed Composition for the Production of Softer Fats

From the supplements used in Example 1 the following fatty acid profile was obtained.

Table 2: Mean fatty acid profiles of control and fat-modified dairy products

Fatty Acid	Control % by wt of total fatty acids	Fat-Modified
Butyric (4:0)	5.7	5.5
Caproic (6:0)	2.7	2.5
Caprylic (8:0)	2.9	1.3
Capric (10:0)	2.8	2.3
Lauric (12:0)	3.3	2.3
Myristic (14:0)	10.0	6.7
Palmitic (16:0)	25.9	15.5
Stearic (18:0)	11.7	14.3
Oleic (18:1)	22.8	35.3
Linoleic (18:2)	1.5	6.9
Linolenic (18:3)	0.7	2.2
Unidentified	10.0	4.2

Example 3: Feed Composition for the Production of Harder Fats

Refer to Figure 2, which illustrates the role of protected lipids, in altering the proportions of fatty acids in milk. In this example, the proportion of C18:0 increased and there was a decrease in C18:1, thereby resulting in a substantial increase in both the melting point of milk fat and its hardness.

Example 4: Protected Lipid Preparation

Cottonseed was coarsely comminuted in a hammer mill and mixed with ethoxyquin (150ppm on an oil basis). This material was then mixed with water to produce a slurry and, after emulsification of the oil and protein in a colloid stone mill, the caustic soda was

added to solublise the oilseed protein. The protein constituents of the homogenised oil seed were cross-linked with 37% (w/v) formaldehyde at the rate of approximately 1.5-3g formaldehyde per 100g crude portion to form a gel which was then dried in a pneumatic drier with an average hot air temperature of 300°C to complete the reaction and produced a protected lipid that was 60-90% resistant to metabolism in the rumen *in vitro*.

(a) Protected Canola Lipid.

Canola lipid was emulsified with protein, and the protein constituents of the homogenised oil seed were cross-linked with formaldehyde at a rate of approximately 2.5g formaldehyde per 100g crude portion producing a supplement that was 75% resistant to metabolism in the rumen *in vitro*.

(b) Protected Cotton Lipid.

Cotton lipid was emulsified with protein, and the protein constituents of the homogenised oil seed were cross-linked with formaldehyde at a rate of approximately 3.0g formaldehyde per 100g crude portion producing a supplement that was 80% resistant to metabolism in the rumen *in vitro*.

(c) Protected Cotton - Soybean/Tallow Lipid.

Canola/Soybean/Tallow lipid was emulsified with protein, and the protein constituents of the homogenised oil seed were cross-linked with formaldehyde at a rate of approximately 2.5g formaldehyde per 100g crude portion producing a supplement that was 80% resistant to metabolism in the rumen *in vitro*.

(d) Protected Soybean - Fish Oil Lipid.

Soybean-fish oil was emulsified with protein, and the protein constituents of the homogenised oil seed were cross-linked with formaldehyde at a rate of approximately 2.5g formaldehyde per 100g crude portion producing a supplement that was 75% resistant to metabolism in the rumen *in vitro*.

Example 5: Protection of Protein Supplements

Protected protein was prepared by spraying 37% (W/V) formaldehyde at the rate of between 0.05 and 0.8g formaldehyde per 100g crude protein into a rapid mixing device containing milled oil seed meal (38% crude protein). This material was then transferred to sealed storage for 10 days to give a supplement 50-70% resistant to proteolysis in the rumen.

(a) Protected Sunflower Protein.

Protected sunflower protein was prepared by reacting approximately 0.7g formaldehyde per 100g with milled sunflower seed meal (38% crude protein, 2% crude lipid), producing a supplement 65% resistant to proteolysis in the rumen.

(b) Protected Canola Protein.

Protected canola protein was prepared by reacting approximately 0.5g formaldehyde per 100g with milled canola seed meal (38% crude protein, 2% crude lipid), producing a supplement 70% resistant to proteolysis in the rumen.

(c) Protected Lupin Protein.

Protected lupin protein was prepared by reacting approximately 0.6g formaldehyde per 100g with milled lupin seed meal (38% crude protein, 5% crude lipid), producing a supplement 65% resistant to proteolysis in the rumen.

(d) Protected Cottonseed Protein.

Protected cottonseed protein was prepared by reacting approximately 0.3g formaldehyde per 100g with milled cottonseed seed meal (38% crude protein, 2% crude lipid), producing a supplement 75% resistant to proteolysis in the rumen.

Example 6: Protection of Carbohydrate Supplements

Grain was coarsely comminuted in a hammer mill to a particle size of approximately 2.5mm or smaller. Protected carbohydrate was then prepared by spraying 37% (W/V) formaldehyde at the rate of between 0.1 and 3.0 grams formaldehyde per 100g crude carbohydrate into a rapid mixing device containing milled concentrate. This material was then transferred to sealed storage for 10 days to give a protected carbohydrate supplement 30-80% resistant to degradation in the rumen.

(a) Protected Wheat Carbohydrate

Protected wheat carbohydrate was prepared by reacting approximately 1.2g formaldehyde per 100g with milled wheat, producing a supplement 65% resistant to degradation in the rumen.

(b) Protected Barley Carbohydrate

Protected barley carbohydrate was prepared by reacting approximately 1.4g formaldehyde per 100g with milled barley, producing a supplement 70% resistant to degradation in the rumen.

Industrial Applicability

The present invention makes use of nutritional materials protected against rumen degradation, but offers the possibility of altering the fatty acid profile of milk produced from female ruminant livestock. In particular, it describes feed supplements which produce milk with a desired fatty acid composition and are useful in producing products with a range of melting profiles. Practise of this invention can be expected to offer economic benefits irrespective of the type of animal in question.

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Claims

1. A method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method comprises feeding to the ruminant livestock, protected lipid having said desired proportions of fatty acids, such that about 60 to about 90% of said protected lipid is capable of passing through the rumen undigested leaving about 60 to about 90% of said protected lipid available for digestion post-ruminally.

2. The method according to claim 1, wherein 75-90% of protected lipid is capable of passing undegraded through the rumen.

3. A method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions of fatty acids, wherein said method comprises feeding to the ruminant livestock protected lipid having said desired proportions of fatty acids, wherein said protected lipid is produced by the emulsification of lipid with protein in the presence of between about 1.5 grams and about 3 grams of formaldehyde per 100 grams crude portion.

4. The method according to claim 3, wherein the protected lipid is produced by the reaction with between about 2.0 grams and about 3.0 grams of formaldehyde per 100 grams crude portion.

5. The method according to any one of claims 1 to 4, wherein the desired proportions of fatty acids are: C18:1 cis (25-45%w/w); C18:2 (4-15%w/w), including conjugated isomers (0.05 to 5%w/w), C18:3 (1-4%w/w); C20:5 and C22:6 omega fatty acid (1-2%w/w).

6. The method according to any one of claims 1 to 4, wherein the desired proportions of fatty acids are: C16:0 cis (25-35%w/w), C18:0 (20-30%w/w) and C18:1 (20-25%w/w).

7. The method according to any one of claims 1 to 6, wherein the source of lipid is selected from the group consisting of: soybean, cotton, lupin, peanut, sunflower, canola, sesame seed, copra and coconut, palm kernels, linseed, casein, butterfat, lard, fish oils, tung oil, and tallow, or from oil lipid products, including conjugated linoleic acid, derived from oil sources by chemical/biological processes, or a combination thereof.

8. The method according to any one of claims 1 to 6, wherein the source of lipid is yellow grease.

9. The method according to any one of claims 1 to 8, further comprising simultaneously feeding to the ruminant livestock protected protein, together with protected protein, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally.

10. The method according to any one of claims 1 to 8, further comprising simultaneously feeding to the ruminant livestock protected carbohydrate such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen

undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

11. The method according to any one of claims 1 to 8, further comprising simultaneously feeding to the ruminant livestock (i) protected protein, such that such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally, and (ii) protected carbohydrate, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally

12. A method according to claim 11, wherein the source of protein and carbohydrate is plant and includes any one of, or a combination of barley, corn, oats, wheat, rice, millet, triticale, rye, and sorghum, soybean, cotton, lupin, peanut, sunflower, canola, sesame seed, copra and coconut, palm kernels and linseed.

13. The method according to any one of claims 1 to 12, further comprising, feeding to the ruminant livestock any other source of processed or unprocessed feedstuff.

14. The method according to any one of claims 11 to 13, wherein the protected carbohydrate, protected protein and/or protected lipid is included in the ration at about 10-45% during the lactation phase.

15. Milk fat obtained from a ruminant fed according to the method of any one of claims 1 to 14.

16. The milk fat of claim 15, wherein said milk fat is comprised of nutritionally desirable soft fats.

17. The milk fat of claim 15, wherein said milk fat is comprised of hard fats.

18. The milk fat of any one of claims 13 to 17, wherein said milk fat is used in the production of milk based products.

19. The milk fat of any one of claims 13 to 18, wherein said milk based products include: milk, butter, cheese, yoghurt, chocolate or infant formula.

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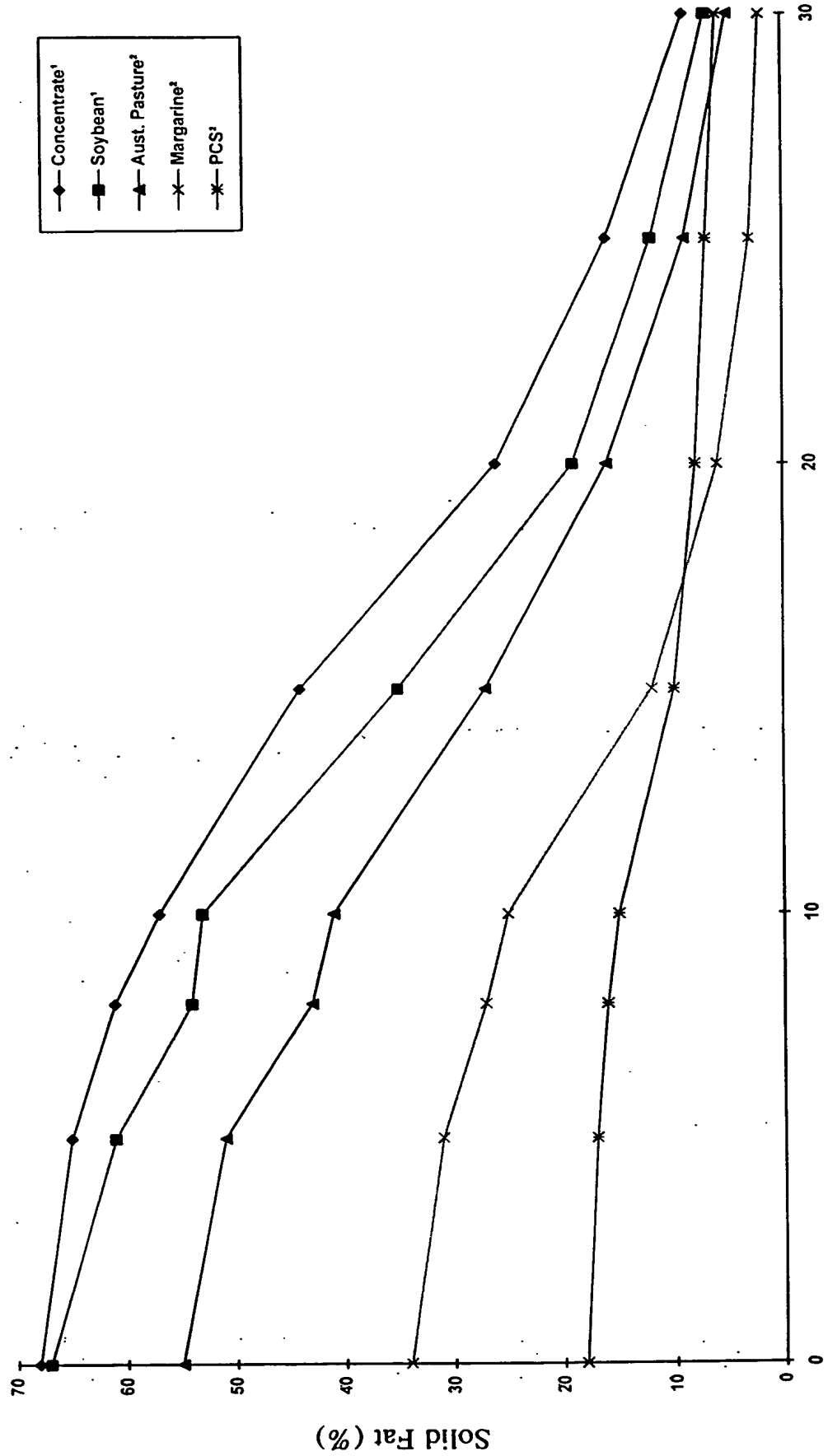
Concentrate¹
Soybean¹
Aust. Pasture²
Margarine²
PCS³
Solid Fat (%)
70
60
50
40
30
20
10
0
0
10
20
30
Temperature °C

Figure 1

Figure 2